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Crude Fibre Determination of *Malva sylvestris* L. and Evaluation of its Faecal Bulking and Laxative Properties in Rats

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Abstract

Malva sylvestris L. or as it is widely known, the common mallow, is a renowned medicinal plant which can be found growing in abundance in Europe, North Africa and Asia. The percentage crude fibre content present in *M. sylvestris* samples collected from Malta was studied. Variation in crude fibre content with time and location was also considered in this study. Results showed that the percentage stem fibre content (27.61%) significantly ($p<0.001$) supersedes the percentage content in leaves (6.49%). These values confirm results attained by various authors in similar studies. Statistical analysis demonstrated that the down trending in percentage leaf fibre content observed over the 3-month study period was only statistically significant ($p<0.01$) for one locality (N=4). In the latter, a significant ($p<0.01$) dip in fibre content was noted for samples collected in April. Conversely, the percentage stem fibre content increased as the plant matured. This observed increase in stem fibre was statistically significant ($p<0.05$) for two of the localities (N=4) studied.

The faecal bulking competency of this fibrous plant was studied in male Sprague-Dawley rats. Throughout the study, the control group was fed fibre-free pellets. Whilst the test group, was fed pellets containing 100 g/kg *M. sylvestris* ground stems. An increase in faecal weight by 105% and 86% was observed in the test group when compared to the fresh and dry faecal weights of the control group. These findings confirm faecal bulking properties and support the potential use of this plant species as a complementary and alternative medicine in the treatment of constipation.

Keywords: Bulk laxative, crude fibre, faecal bulking, Malta, *Malva sylvestris*

1. Introduction

1.1 Use of Plants as Complementary and Alternative Medicine

The medicinal value of plants has been recognized worldwide for thousands of years (Brown, 1995; Chevallier, 1996; J. Barnes, Anderson, & Phillipson, 2007). Their use in treating ailments is the earliest form of healthcare known to us (Kunle, Egharevba, & Ahmadu, 2012) and it is currently estimated that more than 100 million Europeans make use of complementary and alternative medicine (CAM), which encompasses phytotherapy (European Information Centre for Complementary and Alternative Medicine [EICCAM], 2008; World Health Organization [WHO], 2013). Numbers are even bigger for CAM users in Africa, Asia, Australia and North America (Ernst, 2000; P. M. Barnes & Bloom, 2008; WHO, 2013), with up to 80% of the population in low and middle income countries depending on traditional medicine for their primary health care needs (WHO, 2004; Tiwari, 2008).

Malva sylvestris was grown for its medicinal qualities in Roman times (Brown, 1995) and the young leaves and shoots have been consumed since at least the 8th century BCE (Chevallier, 1996; Barros, Carvalho, & Ferreira, 2010). In the middle-ages it was known as a "cure-all" and was used primarily for its soothing properties (Brown, 1995). Whilst locally, *M. sylvestris* known as "il-Ħubbejża" or "Ħobbejża" in Maltese, was boiled and used as a poultice to suppress inflammation (Lanfranco, 1975) and for "purifying the blood" (Lanfranco, 1992).

1.2 Laxative Properties

A generous number of authors suggest that *M. sylvestris* has a laxative effect on the bowels when ingested. The plant has been described as having varying laxative properties depending on which portions of the plant are utilized and the type of preparation employed (Gasparetto, Martins, Hayashi, Otuky, & Pontarolo, 2012). It has also been classified as an intestinal stimulant (Boulos, 1983) and an excellent laxative when used in young children (Lim, 2014). Even Cicero (106-43 BCE), in one of his letters reveals that a decoction of Mallow (*M. sylvestris*) and Chard (*B. vulgaris*) gave him copious diarrhoea (Woodville, 1810; Debuigne, 1988/2004). Yet other literature attribute the plant's laxative properties to the ingestion of large quantities of plant matter (Brown, 1995).

1.3 Fibre Crop

M. sylvestris is a member of the family Malvaceae, which encompasses plants that are typically fibrous. *Hibiscus cannabinus, Urena lobata* and *Hibiscus sabdariffa* are species from this family which are cultivated specifically for their stem fibre (De Rougemont, 1990). Although *M. sylvestris* is not considered for cultivation, it is still highly fibrous and may be classified as a bast fibre crop (Detmers, 1909). Apart from possessing a fibrous stem, *M. sylvestris* is also renowned for its mucilage content (Brown, 1995; Chevallier, 1996) which is a constituent of dietary fiber (American Association of Cereal Chemists [AACC], 2001) and it is thought that these proportions of fibre may be a contributing factor to the plant's laxative effect (Kallas, 2010).

1.4 Fibre Consumption

The current interest in fibre as a dietary constituent may be attributed to British physicians (1950s-1970s) who reported their observations with respect to health status and low- versus high-fibre diets (Dreher, 2001; De Vries, 2003). It was noted, that diseases suffered by populations who consumed high fibre diets were quite different to those suffered by individuals whose diets were comparatively low in fibre content (A. H. Ensminger, M. E. Ensminger, Konlande, & Robson, 1994). Constipation, being one of the conditions, was and still is associated with a low fibre diet (De Vries, 2003).

Knowingly or not, plant fibre has been used to relieve constipation for millennia (Heaton, 1997). Bulking and softening effects of fibre are largely attributed to its ability to absorb and retain water in the bowel, thereby adding volume to the stool (Dwyer, Goldin, Gorbach, & Patterson, 1978). Fibre may also help correct constipation by undergoing microbial fermentation to short chain fatty acids (A. H. Ensminger et al., 1994). This increases biomass, hence increasing stool bulk (Eswaran, Muir, & Chey, 2013). Consumption of fibre in recommended amounts also tends to normalize intestinal transit time. Thereby combating both constipation and diarrhoea (Slavin & Jacobs, 2010) and reducing the incidence of bowel-related disorders (Dwyer et al., 1978).

This study is aimed at determining the crude fibre content present in the leaves and stems of the local *M. Sylvestris* and its variation over time. The faecal bulking competency of this fibrous plant in rats is also considered in this study. Even though heavily documented that *M. sylvestris* is laxative, supporting clinical evidence is scarce. To classify as a faecal bulking agent *M. sylvestris* should produce the desired effect of increasing faecal weight and faecal water content (Hamilton, Wagner, Burdick, & Bass, 1988; Nyman & Asp, 1982; Nyman, Schweizer, Tyren, Reimann, & Asp, 1990; Bravo et al., 1992).

2. Method

2.1 Chemical Investigation

Stem and leaf samples of *M. sylvestris* were collected from four different locations in Malta (Mosta, Mriehel, Naxxar and Birkirkara) during the first week of February, March and April. The leaves were identified. Samples were cleaned under running water and were then cut to uniform size and dried in an over at 40°C for 24hrs. The Association of Official Analytical Chemists [AOAC] official method 962.09 for crude fibre determination was adopted (Cunniff, 1995; Kirk & Sawyer, 1991). Briefly, the dried plant material was ground to uniform fineness and was passed through a stainless steel mesh sieve (No. 20). Fat was removed from approximately 2-3g of dried ground sample using Soxhlet extraction with petroleum ether. The defatted sample was digested with 1.25% H_2SO_4 and 1.25% NaOH solutions. After digestion, the sample was dried in an oven at 130°C for 2 hours and then ignited at 600°C for 30 minutes. The loss in sample weight on ignition and the weight of the ground sample before defatting were utilized to determine the % crude fibre content.

$$\text{\% Crude Fibre in Ground Sample} = \frac{\text{Loss in Weight on Ignition X 100}}{\text{Weight of Ground Sample}} \tag{1}$$

2.2 Pharmacological Investigation

Eleven male Sprague-Dawley rats having an initial mean weight of 370 g were randomly divided into two groups of 5 (control group) and 6 (test group) subjects. The subjects were housed separately and kept on a standard 12h light-dark cycle. Room temperature and humidity level were controlled and kept at 20 ± 1°C and 55 ± 5% RH respectively. Food pellets prepared in the laboratory and water were provided to both groups *ad libitum*. The diets were similar in composition (Table 1) except for the fibre content. Whereby, a 100g portion of corn starch was replaced with an equal quantity of *M. sylvestris* ground stems in the experimental diet. Body-weight and pellet consumption were recorded daily. Following an adaptation phase (4-days), faeces were collected daily during a 5-day test phase. Collected faeces were weighed (fresh weight) and dried in an oven at 104° C for 24h and reweighed (dry weight).

Table 1. Composition of Diets (g/kg) (Bravo et al., 1992)

Ingredients	Control Diet (g/kg)	Experimental Diet (g/kg)
Casein + 6g DL-methionine	150	150
Sucrose	100	100
Olive and sunflower oil	50	50
Mineral & vitamin mixture*	15	15
Corn starch	620	520
M. sylvestris (dried)	-	100

**Composition.* Vitamin A 50000IU; Vitamin E 300IU; Vitamin C 600mg; Folic acid 4000mcg; Vitamin B_1 15mg; Vitamin B_2 17mg; Niacinamide 200mg; Vitamin B_6 20mg; Vitamin B_{12} 60mcg; Vitamin D 400IU; Biotin 300mcg; Pantothenic acid 100mg; Calcium 11.62g; Phosphorous 1250mg; Iodine 1500mcg; Iron 180mg; Magnesium 1g; Copper 20mg; Zinc 150mg; Manganese 25mg; Potassium 400mg; Chloride 363mg; Chromium 250mcg; Molybdenum 250mcg; Selenium 250mcg; Vitamin K_1 250mcg; Nickel 50mcg; Silicon 100mcg; Vanadium 100mcg – per Kg diet.

3. Results

3.1 Crude Fibre Content

The percentage crude fibre content was determined using the equation presented in section 2.1 and the data generated were computed and tested for significance using One-way ANOVA followed by Bonferroni post-hoc test (XLSTAT 2014.4.04, Addinsoft Inc.).

Table 2. Mean (± SEM) Percentage Fibre Content by Location, Plant Parts and Time

Location		Fibre content over time (%)			
		February	March	April	Average
Mosta	*Leaves*	*6.58±0.11*	*6.43±0.13*	*5.66±0.08*	*6.22±0.29*
	Stems	21.34±*0.49*	22.64±0.07	28.83±0.26*	24.27±2.31
Mriehel	*Leaves*	*7.16±0.11*	*6.62±0.25*	*6.16±0.09*	*6.65±0.29*
	Stems	28.76±0.95	29.66±*0.18*	32.11±0.13	30.18±1.00
Naxxar	*Leaves*	*7.02±0.13*	*7.79±0.13*	*5.63±0.38***	*6.81±0.63*
	Stems	32.20±0.45	32.61±0.52	35.37±0.60	33.39±1.00
Birkirkara	*Leaves*	*6.06±0.23*	*6.54±0.08*	*6.26±0.09*	*6.29±0.14*
	Stems	19.38±0.32	18.73±0.25	29.67±2.77***	22.59±3.54

* $p<0.05$, ** $p<0.01$, *** $p<0.001$ with respect to other time-points for the same locality and category.

3.2 Faecal Bulking

The data obtained were computed and tested for significance using two-tailed Student's *t*-test, assuming equal variance and two-way ANOVA for the comparison of trends for the test and control groups with time (XLSTAT 2014.4.04, Addinsoft Inc.).

Table 3. Mean Weights (± SEM) in Grams for the Control and Test Groups

	Mean fresh faecal weights (g/d)	Mean dry faecal weights (g/d)	Mean faecal water content (%/d)	Mean feed intake (g/d)	Mean weight gain (g/d)
Control (N=5)*	*0.91±0.09*	*0.67±0.05*	*25.20±1.30*	*18.37±0.63*	*1.70±0.52*
Test** (*N*=6)	1.86±0.12‡	1.24±0.10‡	32.60±1.52	17.63±*0.84*	1.66±*0.48*

*Control subjects were on a fibre-free diet, **Test subjects were fed pellets containing *M. sylvestris.*

‡ p<0.05 with respect to the control group.

4. Discussion

4.1 Chemical Investigation

The average percentage fibre content present in leaf samples of the local *M. sylvestris* collected over a period of three months (February – April) from four different locations was compared and found to be higher than values obtained in a similar study by Tabaraki, Yosefi, and Gharneh (2012). The leaf samples in the latter study were collected from three different locations in Iran between July and September and the fibre content per location was found to be 2.95%, 3.76%, 5.00% respectively. However, fibre values attained for the local plant were much lower than those obtained in a study performed by Hussain et al. (2013). Whereby, a crude fibre value of 12.18% was recorded for *M. sylvestris* leaf samples collected from Pakistan. This variation in fibre content levels may be attributed to changes or differences in environmental factors. Seasonal variations, altitude, weather conditions, light and soil constituents were all found to influence nutrient content levels in plants (Laycock & Price, 1970; Lascano, Schmidt, & Barahona, 2001). Environmental factors such as moderate water stress may interrupt the progression towards maturity and therefore maintain low plant fibre (Buxton, 1996; Grant, Kreyling, Dienstbach, Beierkuhnlein, & Jentsch, 2014). On the other hand, high summer temperatures promote maturation and an increase in plant fibre content (Mueller & Orloff, 1994; Buxton, 1996; Moore & Jung, 2001). Various authors describe how environmental variations do not only impact plant fibre content but also affect other plant constituents. A variation in the concentration of these constituents such as protein and minerals is anticipated, if these had to be assessed (Laycock & Price, 1970; Murphy, Colucci, & Padilla, 1999; Grant et al., 2014). Example, rain exposure may result in leaching of certain nutrients such as protein, phosphorus and ash. On the other hand, crude fibre is not leached and consequently increases in content as leaching progresses (Laycock & Price, 1970).

Throughout the three month study period the percentage stem fibre content was always significantly ($p<0.001$) higher in all four localities when compared to the leaf fibre fraction. These findings concur with other studies which indicate that the fibre content found in plant stems is significantly greater than the leaf portion (Murphy et al., 1999). The latter having more protein and digestible energy than the fibrous stem. This difference in fibre content was expected since this plant species tends to maintain a shrub-like arrangement where the supporting stems and branches have more structural constituents when compared to the leaves.

The variation in fibre content over time followed a similar pattern for all four locations studied. Statistical analysis demonstrated that the down trending observed in percentage leaf fibre content was not statistically significant ($p>0.05$) except in one of the localities (N=4) studied. In the latter, a significant ($p<0.01$) dip in fibre content was noted for samples collected from Naxxar in April. Concluding, that overall, the leaf fibre fraction remained relatively constant showing minimal variation over the study period. Conversely, the stem fibre content results increased with plant maturity. The difference in stem fibre content between February and April was found to be statistically significant ($p<0.05$) in two of the localities (N=4) studied. These results coincide with studies conducted by other authors where it was observed that as plants mature their fibre fraction increases (Murphy et al., 1999; Moore & Jung, 2001; Grant et al., 2014).

The difference in stem fibre content for samples collected from different localities throughout the 3-month study period was found to be statistically significant ($p<0.01$) when fibre values for Naxxar were compared to Mosta and Birkirkara. Whereby, Naxxar samples gave the highest fibre fraction when compared to other localities. However, a constant significant difference in stem fibre content was not observed when the other localities were compared. Leaf samples followed a similar pattern, with samples from Naxxar exhibiting the highest fibre fraction. Nonetheless, the differences observed were not statistically significant for any two locations throughout the 3-month study period. The only significantly ($p<0.01$) different leaf sample being that collected from Naxxar, in March. Consistency in fibre content results for the majority of localities was expected. Reason being, Malta is a small island and only a few kilometres separate the four localities mentioned in this study. Thus, changes in environmental factors which are known to influence fibre content are typically minimal from one location to another. Furthermore, the type of soil in this area of the Island is also similar, being mainly calcareous (Vella, 1999).

4.2 Pharmacological Investigation

The introduction of *M. sylvestris* to the basal diet had no impact on the overall growth pattern or the mean food consumed by the rats during the 5-day test phase. The weight gain observed was similar for both groups and no significant difference was noted in the amount of pellets consumed by the two groups. These findings coincide with results attained for a similar study conducted on rats using apple pulp as a source of fibre (Bravo et al., 1992).

A statistically significant ($p<0.05$) increase in both fresh and dry faecal weights was observed when the experimental diet containing *M. sylvestris* was consumed. An increase of 105% and 86% in fresh and dry faecal weights respectively was noted for fibre-fed rats when compared to the control group. These results are comparable to those obtained in similar studies carried out utilizing high-fibre containing diets (Nyman & Asp, 1982; Nyman et al., 1990; Bravo et al., 1992).

This increase in faecal weights and water content may be attributed to the fibre components present (Vuksan et al, 2008) that exert a bulking effect on faeces (Prynne & Southgate, 1979) and which were found to increase faecal mass (Alvarado, Pacheco-Delahaye, & Hevia, 2001) through a combination of mechanisms. The water-binding properties of fibre that is resistant to bacterial fermentation in the large intestine increases bulk and stimulates colonic movement. Whilst extensively fermented fibre gives rise to microbial growth which increases faecal bacterial mass and faecal weight. Increasing bulk in the large intestine shortens the transit time. Thereby, decreasing water absorption from the colon and subsequently producing wetter stools. Additionally, gas produced as a by-product of fermentation which becomes trapped within the intestinal content also adds to the bulk (Cummings, 2001).

Thus, the mechanism by which fibre attains its faecal bulking activity is highly dependent on the ratio of fermentable to non-fermentable fibre present in the source. It has been shown that faecal output is greater for sources that are higher in non-fermentable fibre such as wheat fibre than for cabbage fibre which contains a high proportion of fermentable fibre (Cummings & Stephen, 1980; Stephen & Cummings, 1980). Increase in faecal weight was also attributed to unfermented plant fibre when wheat was the fibre source (Chen, Haack, Janecky, Vollendorf, & Marlett, 1998).

These conclusions may explain the high dry faecal weight values obtained for fibre-fed rats (1.24g/d).Values attained in this study were higher than when wheat bran was the fibre source (0.72g/d) (Nyman & Asp, 1982), suggesting that *M. sylvestris* is rich in non-fermentable fibre.

5. Conclusion

The average fibre content of the local *M. sylvestris* was found to vary between 6.49% in the leaves and 27.61% in the stems. Thereby confirming results obtained in similar studies conducted on other plant species which indicate that the stem fibre content in plants is significantly greater than the leaf portion. It was also shown in this study that the percentage fibre content in the stems increases as the plant matures.

Furthermore, it was demonstrated that *M. sylvestris* significantly increases faecal weight when introduced in rat feed, thereby confirming faecal bulking activity. Results attained in the pharmacological investigation are expected to be reconfirmed if tested in human subjects since a correlation has been established between this rat model and humans (Nyman et al., 1990).

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